Chapter

Bioactive Secondary Metabolites of Wild *Antheraea mylitta* Silkworm Cocoons

Sayed Iqbal Ahamad, Kari Neetha and Shyam Kumar Vootla

Abstract

The wild silkworm *Antheraea mylitta* is grown and cultivated in several parts of India ranging from Bihar to West Bengal and several parts of Telangana. The wild silkworm rearing has been a source of income for the tribal populations who rely on it as income source; the intervention of government agencies has increased the cultivation. Our research involves understanding the secondary metabolites in the silkworm Cocoons and elucidating how the pupa survives the harsh environment during pupal diapause of the insect. We have realized the role of insect repellent compounds and other metabolites and their interaction with the insect. Wild silkworm Cocoons are the specialized natural structures constructed by *Antheraea mylitta* silkworms. They are the protein composites of sericin and fibroin as a structural material. The silkworm cocoons are presumed to be evolved structures through the course of evolution over millions of years. This chapter focuses on Biophysical analysis of chemical compounds, proteins and other secondary metabolites traced in the Wild *Antheraea mylitta* Tasar cocoons which are predicted to be the key factors to achieve the unique structural and chemical barriers to protect the pupa within the cocoons.

Keywords: *Antheraea mylitta*, Bioactive compounds, Metabolites, Sericin, Tasar Silkworms

1. Introduction

In wild silkworms host plant specificity is achieved due to the co-evolution of host plants and their monophagous or oligophagous specific herbivorous insect's leads to the accretion of host plant derived allelochemicals in the specific insect cocoons. The plant derived chemicals, play a vital role in the life cycle of the respective phytophagous insects. These bioactive compounds affect the growth, survival, fecundity including behavior of the insects. The economically significant insect cocoons of Tasar silkworm also revealed for biological functions by their secondary metabolites like saponins, flavonoids, terpenoids, tannins and phytosterols sequestered from plant into the larvae to cocoons [1]. The feeding habit and the growing conditions of the silkworms directly influence the chemical composition of the cocoons and the phytochemicals from the host plants of silkworms might be sequestered to cocoons [2]. The secondary metabolites consumed by the silkworms

from the host plants are sequestered with silk proteins and play significant role in cocoon formation [3]. In the cocoons of mulberry silkworms three flavonoid 5-glucosides and many other flavonoids of host plant were identified in the sericin layer in yellow-green cocoon of the Sasammayu silkworms. These flavonoids from silkworm cocoons are proved effective for free radical scavenging, antioxidation, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory action [4] Recently along with mulberry silkworms, the wild non-mulberry silkworms also emerged as commercially significant in textile industry [5] Hence, in present study we focused on the extraction of non-protein active chemical compounds *Antheraea mylitta* cocoons qualitatively and validated by using Fourier Transform-Infrared spectroscopy (FT-IR) and Gas chromatography–Mass spectrometry (GC–MS). The biological activity of the compounds was screened by compared; the phytochemicals from the cocoons were further confirmed in their respective host plants from the earlier reports to elucidate the arthropod-host plant interactions to predict the sequestration of allelochemicals from host plants to the insect cocoons.

As compared to domesticated mulberry silkworm cocoons, the wild silkworms and their cocoons show slightly different combination of morphological, chemical properties and are adapted to cope with harsh natural conditions. By considering this, we selected commercially exploited silkworms of domesticated and wild silkworms to screen the active chemical components from their cocoons and their sequestration patterns from feeding plants to the cocoons by qualitative methods.

The cocoons of most insect larvae are complex structures potentially serving various synchronized functions. The silkworm cocoon is generally presumed to provide the protection to inactive pupa against predation, biodegradation, dehydration etc. Among the most extensively studied cocoons *Bombyx mori* cocoons take the lead. Silk fabric has been valued in numerous cultures for many millennia [6]. As per commercial production of silk *Bombyx mori* silk has been comprehensively investigated. As representative of many holometabolous insects, the silkworm life cycle start as a larva, passing through five larval instars and after the completion of fifth larval instar, the reduction of the juvenile hormone permit the neurosecretory hormone ecdysone to initiate metamorphosis and activate the initiation of the prepupal stage. The prepupa locates the suitable place for cocoon formation, then start to spin the cocoons. The silkworms spun the cocoons around the constricted body of the pupa which uses silk strands secreted from labial glands [7]. Silk strands themselves are polypeptide polymers composed of multiple components-microfilaments of insoluble proteins (fibroin), covered with a soluble adhesive protein (sericin) that provides structural support for the cocoon [8]. Other minor components include small proteins, lipids, and carbohydrates [9].

The wild sericigenous Indian tropical Tasar Silkworm *Antheraea mylitta* is a polyphagous insect feeding on variety of leaves; it has rich genetic resources of forty four races acclimatized to diverse ecological zones. In the course of evolution it has been evolved with many advanced qualities such as disease resistance, silk quality, fecundity and tolerance to various environmental conditions (**Figure 1**). The wild Tasar cocoons are exposed to various biotic and abiotic environmental conditions, hence the quality of the silk, silk proteins and other secondary metabolites of the cocoons are differing than of mulberry silkworm cocoons [10, 11]. Comparatively the domesticated *Bombyx mori* cocoons are soft and delicate, only the hot water treatment swells and partly dissolves the sericin gum, which coats and cements the fibroin filaments together inthe cocoon. But Wild silkworm species including Tasar silkworms are heavily mineralized with calcium oxalates (**Figure 2**) [12]. In addition to this wild cocoon are additionally stabilized by oxidative phenolic tanning, dityrosine cross-linking, and tannins derived from the caterpillar's food

Figure 1.
Antheraea mylitta *Silkworm.*

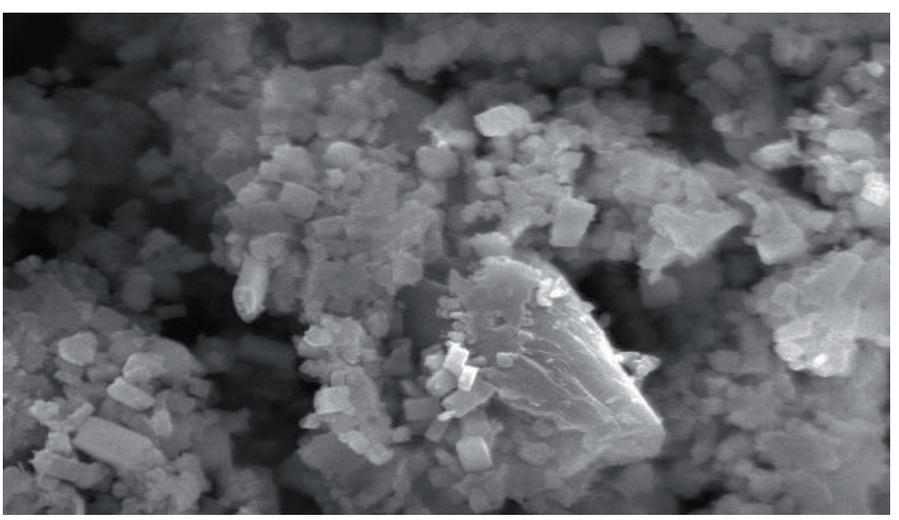

Figure 2.
Scanning Electron Micrograph of calcium oxalate crystals on Antheraea mylitta *cocoons.*

Figure 3.
Antheraea mylitta *Silkworm cocoon spinning.*

Figure 4.
Antheraea mylitta *cocoons collected from forest.*

plant. Mineralization is probably more important than the tanning in making Wild Silk cocoons difficult to soften and reel (**Figure 3**) [13]. The remarkable contrast in the composition of cocoons, the methods of obtaining silk from the cocoons of wild Silkworm with those domesticated *Bombyx mori* makes it complicated. The most important difference lay in the mineralization that very much common in wild Silkworms but absent in *Bombyx mori* and the difference arise from the gluing together of the fibers in mineralized matrix of wild silk cocoons made them moisture resistant (**Figure 4**) [14].

2. Bioactive silk proteins

The cocoons of the mulberry silkworm are composed of two types of proteins: fibroins and sericins. The fibroin is the core protein constitutes 70% of the cocoon and is a hydrophobic glycoprotein secreted from the posterior part of the silk gland. The expression of fibroin protein and P25 genes are transcriptionally regulated during larval development in both *Bombyx mori* and *Antheraea mylitta*. The fibroin protein is semi-crystalline in nature, showing of two phases, i. e greatly crystalline β-sheeted phase and a lesser or non-crystalline phase. The hydrophilic sericin proteins constitute about 20–30% of the cocoon which is hot water-soluble glycoproteins and hold the silk fibers together form the ecologically stable sericin-fibroin composite cocoon structure. The glue like sericin protein biosynthesized and secreted in the middle region of the silk gland. It comprises diverse polypeptides ranging from 24 to 400 kDa with high serine content (40%) with considerable amount of glycine (16%). There are three major polypeptides of sericin have been isolated from the silkworm cocoons with the molecular weights ranges from 150, 250 and 400 kDa. The sericin residues are partially unfolded with 35% β-sheet and 63% random coil, without α-helical structures. In addition to these major proteins, the low molecular weight hydrophilic proteins are also reported in the cocoons. The seroin protein is the product of a discrete gene that is expressed exclusively in the middle and the posterior part of the silk glands [15–20].

Indian tropical wild *Antheraea mylitta* silkworms are of Saturniidae respectively. Silkworms produce delicate twin thread of silk protein fibroin, which is coated by glue like hydrophilic sericin protein (**Figure 5**). During pupation silkworms spin the cocoons to protect the inactive pupae. The silk proteins are synthesized by silk gland cells and stored in the lumen of the glands. The sericin protein is biosynthesized in the middle silk gland of the mature silkworm larvae, which constitutes 25–30% of silk proteins [21]. It is a water-soluble globular protein family whose molecular mass ranges from 10 to 310 kDa [22]. Naturally sericin is responsible for adhere both the fibroin filaments to maintain the structural integrity of the cocoon. The cocoon of *Antheraea mylitta* has three major fractions of sericin of which the lower fraction is around 70 kDa, the middle fraction is approximately 200 kDa, and the higher fraction is more than 200 kDa. The peduncle of Tasar silkworm *Antheraea mylitta* has a 200-kDa sericin protein, possesses serine, glutamic acid, glycine, tyrosine and threonine as predominant amino acid residues. The Serine (~39%) is the principal amino acid. The *Antheraea mylitta* silk sericin is biochemically distinct from *Bombyx mori* having lower percentages of serine and tyrosine. During degumming process of silk textile industry, sericin is removed as waste from fibroin to make silk fibers more lustrous, soft, smooth, white, and dye able. The global discarded sericin constitutes approximately 50,000 tons out of the 1 million tons of fresh cocoons annually [15]. Silk sericin of *Bombyx mori* is one of the most researched proteins. Presently sericin can be used in food, cosmetics, pharmaceutical products and the preparation of biomaterials. The sericin proved as antioxidant, anticoagulant and anti-wrinkle agent. It is also reported to suppress tumor growth and to reduce oxidative stress [23–25].

The domesticated *Bombyx mori* silk sericin contains 18 amino acids including polar amino acids such as 32% serine and 17% aspartic acid gives higher hydrophilic property and processing ability [26]. In contrast to this Tasar silk sericin contains 19% serine contents. The mulberry and Tasar sericins are biochemically distinct due to differences in their amino acid compositions, leading to differences in the immunological responses. Being non-domesticated and wild, Tasar cocoons are more resistant towards environmental stresses such as heat and drying, the sericin coat may contributed for toughness and resistance properties [27].

The silk of domesticated and wild silkworms has a core shell type structure, it is composed of a complex of 3 proteinaceous components: a large heavy chain fibroin (350 kDa) that is linked to a light chain fibroin (25 kDa) by disulfide bonds and another glycoprotein P25 protein (30 kDa) are linked with non-covalent

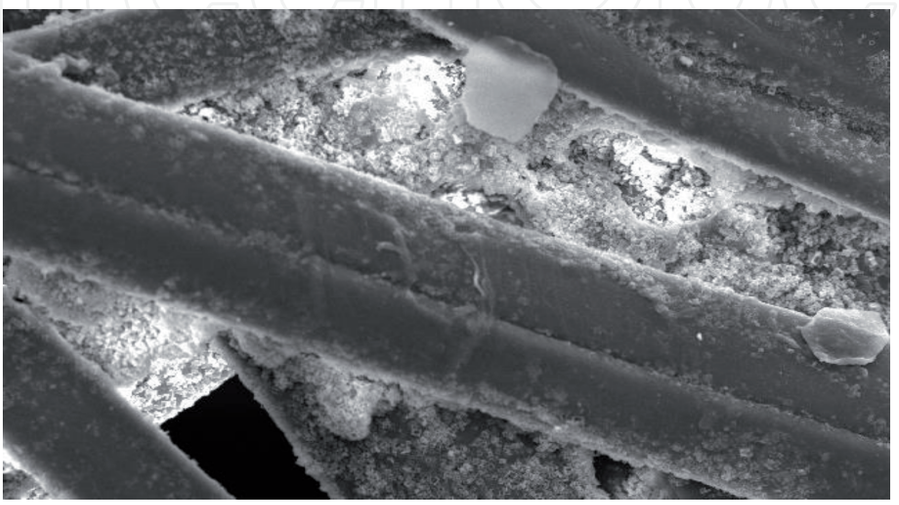

Figure 5.
Scanning Electron Micrograph of Antheraea mylitta *cocoon surface.*

hydrophobic interactions [28]. The molar ratios of Heavy chain, Light chain and P25 are 6:6:1. The heavy chain is hydrophobic in nature and makes crystalline features to the silk fiber, but the Light chain is more hydrophilic and comparatively elastic. The P25 protein is supposed to play a crucial role to maintain the integrity of the complex [29]. Prior to silk fiber formation, the solution of all the three silk proteins secreted from silk glands assembling into double filaments that come out from an exit tube in its spinneret and dry after exposure to air. Consequently core contains anisotropic β-sheet-rich nanocrystals are loosely aligned with the fiber axis and dispersed in an unstructured matrix [30]. Another pair of silk glands secrete glue-like sericins (serine-rich glycoproteins) that coat the fibroin filaments for the cohesion of the cocoon by sticking the twin filaments together. The silk fibers coated with several other proteins are presumed to protect the cocoon against microorganisms and other predators [31, 32].

2.1 Biomedical applications of silk proteins

The silk proteins are biologically versatile molecules in the context of biomedical applications. The silk fibers have been used as sutures for wounds since many centuries, because of its strength, biocompatibility and low immunogenicity [33]. Even the silk fibers spun into yarns and consequently textured via permanent deformation may be used as non-load-bearing spacers in tissue grafts where tissue in-growth is desirable. The cabled yarns have great tunable mechanical properties and therefore potential in load-bearing tissue engineering applications [34]. In addition to these silk foams prepared from fibroin has been used as scaffolds for the attachment and proliferation of fibroblasts *in vitro* condition. The study on biomedical applications of silk protein reveal that the cultured cell colonies were located at the surface of the foam, preferably due to the cell-seeding process due to lacking nutrients inside the foam [35]. Silk-based materials have been used as organic scaffolds for the biomineralization of hydroxyapatite and silica. The silk proteins are considered as potential molecules for a drug release profile and are both reliable and controlled, particularly important in cases where the drugs have undesirable side effects. Silk proteins may find application in drug delivery as drug carriers owing to their biocompatibility and their highly tunable morphologies [36].

Silk protein-based materials have found application as solid supports for potentially expensive enzyme and organometallic catalysts. Silk proteins are capable of forming functional complexes with metal ions. The enzymes can be successfully immobilized by means of covalent linking of the silk protein to the enzyme using conventional cyanogen bromide, azide, diazo or glutaraldehyde methodologies. The aspartate aminotransferase enzyme, calf intestine alkaline phosphatase enzyme and ribonuclease enzymes have been covalently linked to *Bombyx mori* fibroin effectively and were shown their activity efficiently. Many enzymes effectively immobilized through physical entrapment method within the silk films [37–40].

The silk sericin has many biomedical applications as antioxidant, anticancer drug and anticoagulant. The study on macrophage response of silk proteins concludes that silk sericin does not allow inflammatory response when supply in soluble form. But the macrophage activation study of silk sericin reveals that, when attached to fibers induce inflammatory responses [41]. The silk sericin in presence of lipopolysaccharides shows inflammatory reaction by initiating the synthesis of tumor necrosis factor and which is connected with native silk fiber-induced immune responses [42–44]. The explanation could be that coated sericin proteins either provide better adhesion to macrophages or the structural changes of sericin after binding to silk fibers prime the macrophage for consequent stimulation.

The bioconjugation (e.g. polymer-protein) is beneficial because it leads to minimize immunogenicity and improve stability. The biopolymer conjugations with anticancer drug candidates promote tumor targeting efficiency through superior permeability and increase drug retention time. Many investigators have utilized sericin as a natural biopolymer for bioconjugation with various therapeutic proteins, enzymes and polysaccharides. The sericin is the best natural biopolymer which can be conjugated effectively due to the presence of functional surface-active groups (-OH, -COOH, -NH2), which can form covalent linkage with the conjugates. The success of best performance of sericin is due to its hydrophilic nature and low antigenicity and other immunogenic properties and higher half-life period *in vivo* due to filtration by the kidneys to increase retention period [45–49].

The two-dimensional films and three-dimensional matrices of hydrogels and porous scaffolds of sericin proteins have been reported for their better performance. The membranes of sericin are naturally fragile in the dry state. The blending of sericin with water-soluble polymers like polyvinyl alcohol for making films has been investigated. The sericin hydrogels blend with polyvinyl alcohol by irradiation at 40 kGy has been reported. The blended hydrogels show an excellent moisture adsorbing tendency, desorbing and elastic properties with potential applications as soil conditioners to biomaterials for biomedical applications including wound dressings [50, 51].

3. Bioactive Tasar cocoon secondary metabolites

In present research the qualitative analysis of phytochemicals were tested for alkaloids, saponins, steroids, phenols, flavonoids, terpenoids, tannins, fatty acids, carboxylic acids, volatile oils, fixed oils and aldehydes in the methanolic extracts of Tasar cocoons by using standard methods [52] and compiled in **Table 1**. The alkaloids and volatile oils are present in Tasar cocoons whereas terpenoids and tannins are absent. The tests for phenols, fatty acids, carboxylic acids and fixed oils have shown positive reports.

The Tasar cocoon extract contained 14 characteristic GC–MS peaks emerged, which represents various respective chemical components in the extract (**Figure 6**). The Tasar cocoon methanolic extract revealed fourteen characteristic GC–MS peaks which are correlated with FT-IR (**Figure 7**), which represent their respective chemical components in the extract. The chromatogram maximum peak area percentage was observed for 26-Nor-5-cholesten-3.beta-ol-25-one (65%), Oleic acid (9.47%), n-Hexadecanoic acid (7.24%), Stegmasterol (5.93%) and Octadecanoic acid (5.51%). The FT-IR spectral analysis of the compounds of Tasar cocoons are presented in **Table 2** and chemical structures are presented (**Figure 8**). These compounds are reported as potent antimicrobial, anti-inflammatory, anticancer, antioxidant, Hypocholesterolemic in nature respectively [53–59]. Other compounds shown minimum peak area percentage it directly dictates less in concentration. The complete GC–MS analysis and the biological activity of the compounds are presented in **Table 3**.

3.1 4-Methyltridecane and isobutyl alcohol

4-methyltridecane is a branched alkane consisting of tridecane bearing a single methyl substituent at position 4. It has a role as a plant metabolite. It is reported for antioxidant properties [60].

Bioactive compounds	Qualitative tests
Alkaloids	+
Saponins	+
Steroids	+
Phenols	+
Flavonoids	+
Terpenoids	-
Tannins	-
Fatty acids	+
Carboxylic acids	+
Volatile oils	+
Fixed oils	+
Aldehydes	-

Table 1.
Qualitative tests for active compounds from Methanolic extract of Antheraea mylitta *cocoons.*

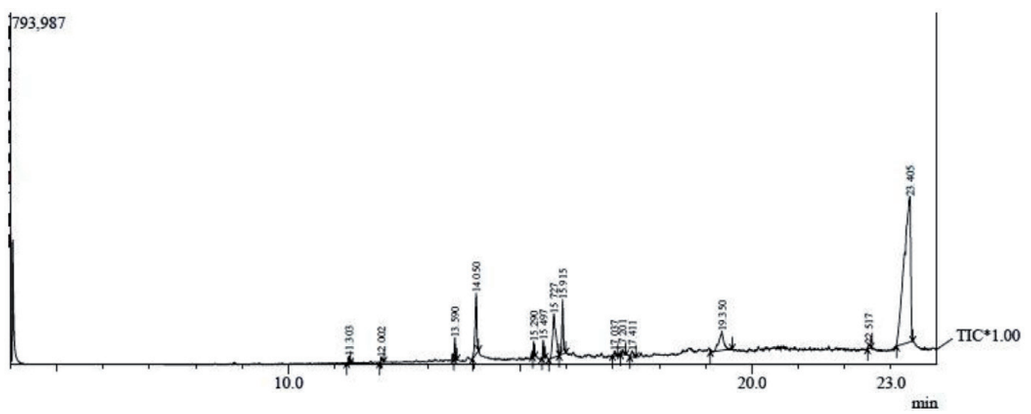

Figure 6.
GC-MS chromatogram of Antheraea mylitta *cocoon extract.*

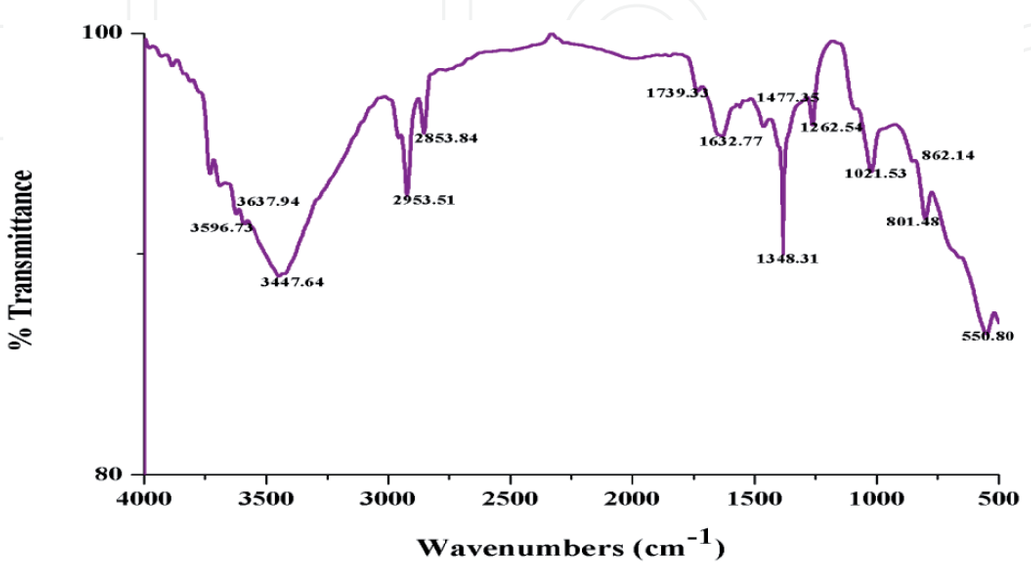

Figure 7.
FT-IR of Antheraea mylitta *cocoon extract.*

FT-IR spectra of Tasar cocoons (Wavenumbers cm-1)	Bonds and Structures	Description
3637.94 3596.73	O-H stretch, free hydroxyl, H-bonded	Alcohols and Phenols
3447.64	N-H stretch	1˚, 2˚ amines, amides
2953.51 2853.84	C-H stretch	Alkanes
1739.33 1632.77	C=O stretch	Amide I
1477.35	C-N Stretching, N-H bending	Amide II
1348.31	N-O symmetric stretch	Nitro compounds
1318.50	C-O Stretch	Alcohols, carboxylic acids, esters, ethers
1262.54 1021.53	C-N Stretching, N-H bending	Amide III (Aliphatic amines)
862.14	O-H bending	Carboxylic acids
801.48	C-Cl Stretch	Alkyl halides
550.80	C-H Stretch	Aromatics

Table 2.
FT-IR spectra of Antheraea mylitta *cocoon extracts.*

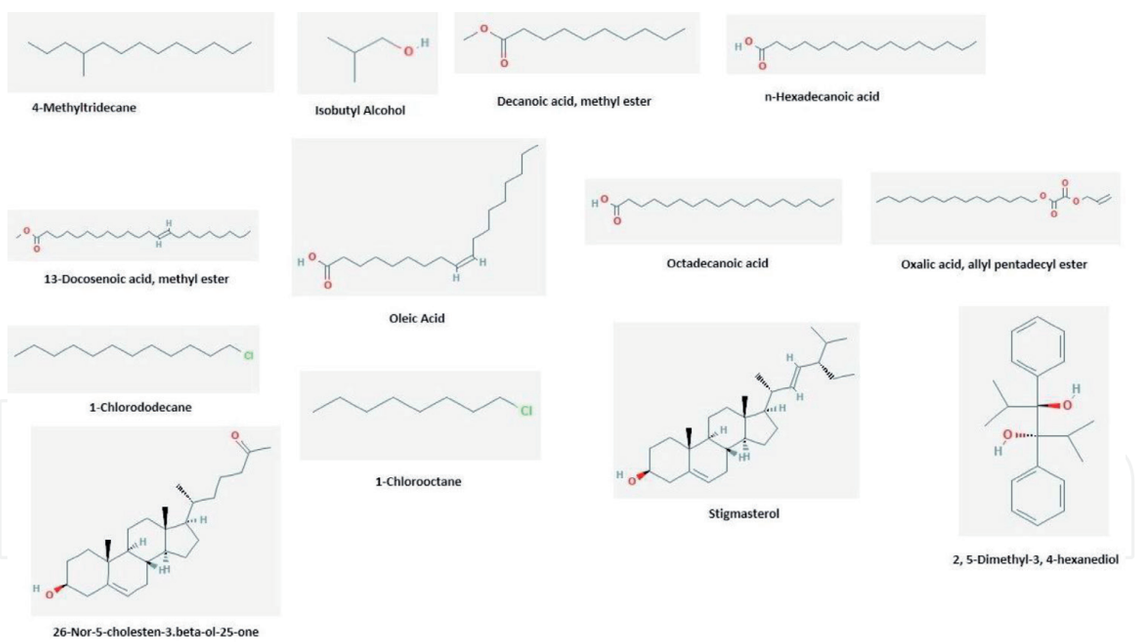

Figure 8.
Chemical structures of Identified Bioactive compounds from Antheraea mylitta *Cocoon Extract.*

Isobutanol is an alkyl alcohol substituted by a methyl group at position 2. In *Saccharomyces cerevisiae* it acts as active metabolite for various physiological functions. This primary alcohol derives from a hydride of an isobutene [61].

3.2 Decanoic acid, methyl ester and N-hexadecanoic acid

Methyl decanoate is a fatty acid methyl ester and a decanoate ester, it is reported for Antioxidant activity, Antibacterial, antiviral, antifungal activity [62].

Name	RT (m)	Area (%)	Structure	Molecular weight	Chemical Nature	Bioactivity
4-Methyltridecane	11.30	0.45	$C_{14}H_{30}$	198	Alkane	Insect predator and Pheromone
Isobutyl alcohol	12.00	0.51	$C_4H_{10}O$	74	Alcohol	Alcohol detoxicant, disinfectant
Decanoic acid, methyl ester	13.59	1.38	$C_{11}H_{22}O_2$	186	Fatty acid ester	Food additive and lubricant, Insecticide
n-Hexadecanoic acid	14.05	7.24	$C_{16}H_{32}O_2$	256	Palmitic acid	Antioxidant, nemeticide and Hypocholesterolemic
13-Docosenoic acid , methyl ester	15.29	0.84	$C_{22}H_{44}O_2$	352	Fatty acid ester	Lubricant and surfactant
Pentanoic acid, 4-methyl, methyl ester	15.49	1.30	$C_7H_{14}O_2$	130	Fatty acid ester	Flavouring agent
Oleic acid	15.72	9.47	$C_{18}H_{34}O_2$	282	Fatty acid	Anticancer, anti-aging and Anti-inflammatory
Octadecanoic acid	15.91	5.51	$C_{18}H_{36}O_2$	284	Fatty acid	Adhesive and sealant
Oxalic acid, allylpentadecyl ester	17.03	0.42	$C_{20}H_{36}O_4$	340	Carboxylic acid ester	-
1-Chlorododecane	17.20	0.54	$C_{12}H_{25}Cl$	204	Alkyl halide	Antitumor, Increase natural killer cell activity
2,5-Dimethyl-3,4-hexanediol	17.41	0.93	$C_8H_{18}O_2$	146	Alcohol	-
Stigmasterol	19.35	5.93	$C_{29}H_{48}O$	412	Steroid	Antioxidant, hypoglycemic and thyroid inhibiting properties, Precursor of progesterone, Antimicrobial, Anticancer, Antiarthritic, Antiasthama, Anti-inflammatory, Diuretic.
1-Chlorooctane	22.51	0.49	$C_8H_{17}Cl$	148	Alkyl halide	Anticancer
26-Nor-5-cholesten-3. beta-ol-25-one	23.40	65.00	$C_{26}H_{42}O_2$	386	Steroid	Antimicrobial, Diuretic, Anti-inflammatory, Anti-asthma

Table 3.
GC-MS Identified Bioactive Secondary Metabolites from Antheraea mylitta *cocoon.*

The hexadecanoic acid is a straight-chain, sixteen-carbon, saturated long-chain fatty acid. N-hexadecanoic acid and it showed significant cytotoxicity against human colorectal carcinoma cells. It is also reported as anti-inflammatory compound [63]. Hexadecanoic acid, methyl ester exhibited antioxidant,

hypocholesterolemic, anti-androgenic, hemolytic, alpha reductase inhibitor activities [64]. The major saturated fatty acid hexadecanoic acid has recently been shown to be neutral in its cholesterolaemic effect. The Palm oil rich with hexadecanoic acid, the consumption has been reported to reduce blood cholesterol in comparison with the traditional sources of saturated fats such as coconut oil, dairy and animal fats [65].

3.3 13-Docosenoic acid, methyl ester

13-Docosenoic acid, methyl ester is a fatty acid methyl ester, that is a flavor-active, volatile, and aromatic compound found in cooked commercial shrimp waste. It is a component of biodiesel formed from *Croton megalocarpus* and *Ceibapentandra* oils that contain trierucin. 13(Z)-Docosenoic acid methyl ester has also been used [66].

3.4 Oleic acid

Oleic acid has been reported to have hypocholesterolemic, antioxidant and lubricating activity [67]. Oleic acid is commonly found in diet. It is a monounsaturated fat which on consumption has been linked with decreased lowdensity lipoprotein cholesterol, and possibly increased high-density lipoprotein cholesterol [68].

3.5 Octadecanoic acid and oxalic acid, allylpentadecyl ester

A C18 straight-chain saturated fatty acid component of many animal and vegetable lipids. As well as in the diet, it is used in hardening soaps, softening plastics and in making cosmetics, candles and plastics. It is a stearic acid ester reported for its antioxidant and anti-inflammatory activity [69]. The alcoholic compound of oxalic acid is reported for antimicrobial preservative [70].

3.6 1-Chlorododecane

1-Chlorododecane belonging to the family of organic halogen compounds. It is hard to dissolve in water but can be mixed with alcohol and ether. This chemical is less health hazard substance than short chain alkyl chlorides. It is used as a solvent, as chemical intermediate to make photographic chemicals, pharmaceuticals, organometallic compounds, surfactants [71].

3.7 2, 5-Dimethyl-3,4-hexanediol and stigmasterol

2, 5-Dimethyl-3, 4-hexanediol extracted from *Phormidium autumnale* is reported for antimicrobial activity [72]. Another steroidal compound Stigmasterol reported for Anti-tumor, Cancer preventive, inhibit intestinal cholesterol absorption, antiinflammatory activity [73].

3.8 1-Chlorooctane

The GC–MS analysis of essential oils obtained from the peel of *Citrus reticulata* was confirmed for1-Chlorooctane as an important compound. It has reported for marked antibacterial and antifungal activities, as evidenced by their zones of inhibition. Among the tested microbiology, the oil was very active against *Bacillus subtilis*, *Aspergillusflavus*, *Escherichia coli* and *Staphylococcus aureus* [74].

3.9 26-Nor-5-cholesten-3.beta-ol-25-one

The steroid compound of cholesten reported for Antimicrobial, Diuretic, Anti-inflammatory, anti-asthmain acetone extract of *Cenchrusse tigerus* [75].

The primary host plant for Tasar silkworm is *Terminalia arjuna* (Combretaceae), it has been reported for its antimicrobial properties and to treat cardiovascular disease [76, 77]. In this study similar phytochemical compounds of *Terminalia arjuna* leaves observed in Tasar cocoons but steroids are not observed in the Tasar host plant [78, 79]. The alkaloids, saponins, steroids, flavonoids, terpenoids, tannins, volatile oils and aldehydes are not observed in the cocoon extract. This observation highlights in addition to the phytochemical sequestrations from host plant to silkworm cocoons, even biosynthesis of the other active compounds by the silkworm larvae takes place during spinning of the cocoons. The mechanism of metabolic pathways for the phytochemical sequestrations or the synthesis of the active compounds observed in the mulberry and wild silkworms need further exploration.

4. Conclusion

The silk fibers and insect extracts have been comprehensively used in folk medicines from thousands of years. In Chinese traditional medicines insects and insect-based products are used for various diseases and ailments. The insects and their products have evolved individually over the track of evolution; naturally insects face numerous biotic and abiotic challenges in their life cycles. Due to microorganisms infested habitats they occupied their success in the survival, infinite numbers and diversity specify the presence of extremely effective immune systems produce powerful antimicrobial, cytotoxic compounds for the parasites and other medicinally valuable chemical compounds. The chemical defense strategies of insects have evolved, including odorous repellents to avoid or to kill or inactivate the defending individual predatory organisms including microorganisms. By considering this, we designated the commercially exploited lepidopteran insect wild Tasar silkworm cocoons to screen the active non-protein chemical components [80].

Naturally, silkworm pupa is enclosed within the cocoons during the metamorphosis from pupa to adult. This is the most susceptible stage for the insects because the immobile pupa is not able to respond to biotic and abiotic threats. Therefore, cocoon provides or modifies the microenvironment of the pupa to ensure the optimal conditions for successful pupation and possesses antimicrobial properties. In comparison to wild silkworms, domesticated mulberry silkworms are more privileged due to their domestication and indoor rearing practices. Hence the structural and chemical composition of wild silkworms is strong enough to face threats during their life cycle. The diverse chemical compounds with strong biological activities were identified in the wild silkworm cocoons compared to mulberry silkworms. The comprehensible mechanism of bioactive compound synthesis and their sequestration from specific host plants to the silkworm cocoons and their function for the silkworm physiology is yet to be explained. The insect specific chemical products including antimicrobial compounds, their biosynthesis strategies and the mechanism of action may take part in the discovery of new drug candidates in the field of biomedical science.

The Bioactive components in addition to the Silk proteins from Tasar cocoons were identified by various methods and their biomedical application was compiled. The qualitative analysis of the extracts was performed to validate the active chemical compounds. The conclusion of our results drawn as the phytochemical of the

host plants sequestrated to cocoons and the biosynthesis of bioactive compounds by the silkworm larvae during spinning of the cocoons to protect pupae during metamorphosis. But the molecular mechanisms or metabolic pathway for phytochemical sequestration or the biosynthesis of the bioactive compounds in the insects need further research. In comparison to mulberry cocoons, non-mulberry cocoons possess the antimicrobial and insect repellent agents, which are presumed to be involved in the direct protection of wild cocoons by microbial decomposition and other insect predators in wild environmental conditions. We conclude the physico-chemical interactions of the cocoons are responsible to protect the inactive pupae during metamorphosis. Further exploration is required for strategic isolation of cocoon protecting active compounds from mulberry and non-mulberry cocoons for biomedical applications.

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Conflict of interest

"The authors declare no conflict of interest."

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References

[1] Bindu PC, Jaisankar P, Hauer F et al (2006) Biological relevance of host plant-derived terpenoid in the cocoons of the tropical Tasar silkworm *Antheraea mylitta*. Biochem Syst Ecol 34(9):698-704

[2] Bharani A, Ganguli A, Mathur LK et al (2002) Efficacy of *Terminalia arjuna* in chronic stable angina: a double-blind, placebo-controlled, crossover study comparing *Terminalia arjuna* with isosorbide mononitrate. Indian Heart J 54(2):170-175

[3] Kumar JP, Mandal BB (2017) Antioxidant potential of mulberry and non-mulberry silk sericin and its implications in biomedicine. Free Radic Biol Med, 108:803-818

[4] Wang HY, Wang YJ, Zhou LX et al (2012) Isolation and bioactivities of a non-sericin component from cocoon shell silk sericin of the silkworm *Bombyx mori*. Food Funct, 3(2):150-158

[5] Arunkumar KP, Tomar A, Daimon T et al (2008) Wild Silk base: an EST database of wild silkmoths. BMC Genomics 9(1):338

[6] Kadolph, Sara J., 2007. Textiles, 10th ed. Pearson Prentice Hall, Upper Saddle River, pp. 76-81.

[7] Asakura, T., Umemura, K., Nakazawa, Y., Hirose, H., Higham, J., Knight, D., 2007. Someobservations on the structure and function of the spinning apparatus in the silkworm *Bombyx mori*. Biomacromolecules 8, 175-181.

[8] Hakimi, O., Knight, D.P., Vollrath, F., Fadgama, P., 2007. Spider and mulberry silkworm silks as compatible biomaterials. Compos, Part B Eng. 38, 324-337.

[9] Gauthier, N., Mandon, N., Renault, S., Bénédet, F., 2004. The *Acrolepiopsis assectella* silk cocoon: kairomonal function and chemical characterization. J. Insect Physiol. 50,1065-1074.

[10] Sayed Iqbal Ahamad M and Shyam KumarVootla.2018, Extraction and Evaluation of Antimicrobial Potential of *Antheraea mylitta* Silk Sericin. Int JRecent Sci Res. 9(1), pp. 23019-23022. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1382

[11] Ahmad, S.E., Kamra, A., Hasnain, S.E. (2004). Fibroin silkproteins from the non-mulberry silkworm *Philosamia ricini* are biochemically and immunochemicallydistinct from those of mulberry silkworm *Bombyx mori*. DNA Cell Biol., 23: 149-154.

[12] Akai, H., & Nagashima, T. (2003). Calcium crystals of cocoon shell from African Gonometa silkmoth (Lasiocampidae). International journal of wild silkmoth & silk, 8, 1-5.

[13] Shamitha, G., & Rao, A. P. (2006). Studies on the filament of Tasar silkworm, Antheraea mylitta D (Andhra local ecorace). Current science, 1667-1671.

[14] Gheysens, T., Collins, A., Raina, S., Vollrath, F., & Knight, D. P. (2011). Demineralization enables reeling of wild silkmoth cocoons. Biomacromolecules, 12(6), 2257-2266.

[15] Couble P, MoineA, GarelA, Prudhomme JC. Developmental variationof a non-fibroin mRNA of *Bombyx mori* silk gland, encoding a lowmolecular silk protein. Dev Biol 1983;97:398-407.

[16] Zhou C, Confalonieri F, Medina N, Zivanovic Y, Esnault C, Yang T, et al. Fine organization of *Bombyx mori* fibroin heavy chain gene. Nucleic Acids Res 2000;28:2413-9.

[17] Kikuchi Y, Mori K, Suzuki S, Yamaguchi K, Mizuno S. Structure of the *Bombyx mori* fibroin light chain-encoding gene: upstreamsequence elements common to the light and heavy chain. Gene1992;110:151-8.

[18] Tanaka K, Kajiyama N, Ishikura K, Waga S, Kikuchi A, Ohtomo K, et al. Determination of the side of disulfide linkage between heavy and light chains of fibroin produced by *Bombyx mori*. Biochim Biophys Acta 1999;1432:92-103.

[19] Mandal, B. B., Ghosh, B., & Kundu, S. C. (2011). Non-mulberry silk sericin/poly (vinyl alcohol) hydrogel matrices for potential biotechnological applications. International Journal of Biological Macromolecules, 49(2), 125-133. https://doi.org/10.1016/j.ijbiomac.2011.03.015

[20] Datta A, Ghosh AK, Kundu SC. Purification and characterization of fibroin from the tropical saturniid silkworm, *Antheraea mylitta*. Insect Biochem Mol Biol 2001;31:1013-8.

[21] Dash, R., Ghosh, S. K., Kaplan, D. L., & Kundu, S. C. (2007). Purification and biochemical characterization of a 70kDa sericin from tropical Tasar silkworm, *Antheraea mylitta*. Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology, 147(1), 129-134. https://doi.org/10.1016/j.cbpb.2007.01.009

[22] Sehnal, F., & Žurovec, M. (2004). Construction of silk fiber core in Lepidoptera. Biomacromolecules, 5(3), 666-674.

[23] Gupta, D., Agrawal, A., & Rangi, A. (2014). Extraction and characterization of silk sericin. Indian Journal of Fibre and Textile Research, 39(4), 364-372.

[24] Zhou CZ, Confalonieri F, Jacquet M, Perasso R, Li ZG, Janin J. Proteins –Structure, Function and Genetics 2001;44(2):119-22.

[25] Marsh, r. E., Corey, r. B., & Pauling, l. (1956). Die struktur von Tussah-seidenotizuber die struktur von beta-poly-l-alanin. In angewand techemie-international edition (vol. 68, no. 6, pp. 218-219). Muhlenstrasse 33-34, d-13187 Berlin, Germany: wiley-v chverlaggmbh.

[26] Altman, G. H., Diaz, F., Jakuba, C., Calabro, T., Horan, R. L., Chen, J.& Kaplan, D. L. (2003). Silk-based biomaterials. Biomaterials, 24(3), 401-416.

[27] Kaur, N., Shafiq, N., Negi, H., Pandey, A., Reddy, S., Kaur, H., & Malhotra, S. (2014). *Terminalia arjuna* in chronic stable angina: Systematic review and meta-analysis. Cardiology research and practice, 2014.

[28] Smritikana Biswas, PranoyMitra. (2017). Comparative studies on the antimicrobial activity of *Terminalia arjuna* and *Aloe vera* against community associated drug resistant *Staphylococcus aureus* in pus from carbuncles of adults. Journal of Innovations in Pharmaceutical and Biological Sciences, 4 (4), 153-157

[29] Desai, D., &Chanda, S. (2014). Pharmacognostic Study and Physicochemical Analysis of Leaves of *Terminalia arjuna*. Pharmacognosy Journal, 6(6), 15-19.

[30] Ahamad, M. S. I., Neetha, K., &Vootla, S. K. (2020). Non-protein Chemical Compounds from Lepidopteran Insect Cocoons. In Natural Materials and Products from Insects: Chemistry and Applications (pp. 137-156). Springer, Cham.

[31] Aramwit, P., Siritientong, T., Srichana, T., 2012. Potential applications of silk sericin, a natural protein from textile industry by products. Waste Manag. Res. 30, 217-224.

[32] Akai, H. (1997). Anti-bacterial function of natural silk materials. Int. J. Wild Silkmoth& Silk, 3, 79-81.

[33] Vollrath, F., Barth, P., Basedow, A., Engström, W., & List, H. (2002). Local tolerance to spider silks and protein polymers in vivo. In vivo (Athens, Greece), 16(4), 229.

[34] Kearns, V., Mac Intosh, A. C., Crawford, A., & Hatton, P. V. (2008). Silk-based biomaterials for tissue engineering. Topics in tissue engineering, 4, 1-19.

[35] Tamada, Y. (2005). New process to form a silk fibroin porous 3-D structure. Biomacromolecules, 6(6), 3100-3106.

[36] Hardy, J. G., Römer, L. M., & Scheibel, T. R. (2008). Polymeric materials based on silk proteins. Polymer, 49(20), 4309-4327.

[37] Grasset, L., Cordier, D., & Ville, A. (1977). Woven silk as a carrier for the immobilization of enzymes. Biotechnology and Bioengineering, 19(4), 611-618.

[38] Grasset, L. (1979). Silk: a natural protein for enzyme immobilization.

[39] Cordier, D., Couturier, R., Grasset, L., & Ville, A. (1982). Ribonuclease insolubilization using diazotized silk. Enzyme and Microbial Technology, 4(4), 249-255.

[40] Grasset, L., Cordier, D., Couturier, R., & Ville, A. (1983). Immobolization of alkaline phosphatase on silk using diazo, adsorption, glutaraldehyde, and azide method: Optimum pH and properties of the conjugates. Biotechnology and bioengineering, 25(5), 1423-1434.

[41] Dash R, Ghosh SK, Kaplan DL, Kundu SC. Purification and biochemical characterization of a 70 kDa sericin from tropical Tasar silkworm, *Antheraea mylitta*.Comp Biochem Physiol B: Biochem Mol Biol 2007;147:129-34.

[42] Yamada H, Tsubouchi K. Characterisation of silk proteins in the cocoon fibers of *Cricula trifenestrata*. Int J Wild Silkmoth Silk2001;6: 17-51.

[43] Dash R, Mukherjee S, Kundu SC. Isolation, purification and characterization of silk protein sericin from cocoon peduncles of tropical Tasar silkworm, *Antheraea mylitta*. Int J BiolMacromol2006;38: 255-8.

[44] Soong HK, Kenyon KR. Adverse reactions to virgin silk sutures incataract surgery. Ophthalmology 1984;91:479-83.

[45] Vicent MJ, Duncan R. Polymer conjugates: nanosized medicines for treating cancer. Trends Biotechnol 2006;24:39-47.

[46] Zhang YQ, Tao ML, Shen WD, Zhou YZ, Ding Y, Ma Y, et al. Immobilizationof l-asparaginase on the microparticles of the natural silk sericin protein and its characters. Biomaterials 2004;25:3751-9.

[47] Zhang YQ, Tao ML, Shen WD, Mao JP, Chen YH. Synthesis ofsilk sericin peptides–l-asparaginase (SS–ASNase) bioconjugates and their characterization. J Chem Technol Biotechnol 2006;81:136-45.

[48] Zhang YQ,MaY, Xia YY, ShenWD,MaoJP,Xue RY. Silk sericin–insulinbioconjugates: synthesis, characterization and biological activity. JControl Release 2006;115:307-15.

[49] Anghileri A, Lantto R, Kruus K, Arosio C, Freddi G. Tyrosinase catalyzed grafting of sericin peptides onto chitosan and production of protein–polysaccharide bioconjugates. J Biotechnol2007; 127:508-19.

[50] Yoshii F, Kume T, Makuuchi K, Sato F. Hydrogel composition containingsilk protein. Japan Patent 2000-169736A; 2000.

[51] Wang S, Goto Y, Ohkoshi Y, NaguraM. Structures and physical properties of poly(vinyl alcohol)/sericin blend hydrogel membranes. J Seric Sci Jpn 1998;67:295-302.

[52] Roy, P., Amdekar, S., Kumar, A., & Singh, V. (2011). Preliminary study of the antioxidant properties of flowers and roots of *Pyrostegiavenusta* (Ker Gawl) Miers. BMC Complementary and Alternative Medicine, 11(1), 69.

[53] Ananthi, P., &Kumari, B. D. (2013). GC–MS Determination of Bioactiv Components of Rorippaindica L. International Journal of Chem Tech Research, 5(4), 2027-2033.

[54] Dey, P., Ray, S., Sarkar, M. P., &Chaudhuri, T. K. (2015a). Chemical characterization and assessment of antioxidant potentiality of *Streptocaulonsylvestre* Wight, an endangered plant of sub-Himalayan plains of West Bengal and Sikkim. BMC complementary and alternative medicine, 15(1), 107.

[55] Chisholm, M. G., Wilson, M. A., &Gaskey, G. M. (2003). Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia* Swingle). Flavour and Fragrance Journal, 18(2), 106-115.

[56] GomathiRajashyamala, L., &Elango, V. (2015). Identification of bioactive components and its biological activities of *Evolvulus alsinoides* linn.--A GC-MS study. IJCS, 3(1), 41-44.

[57] Premlata, S., Mourya, K. K., & Padma, K. (2015). Gas chromatography-mass spectrometric analyses of acetone extract of Marwar Dhaman grass for bioactive compounds. Plant Archives, 15(2), 1065-1074.

[58] Parthipan, B., Suky, M. G. T., & Mohan, V. R. (2015). GC-MS Analysis of Phytocomponents in *Pleiospermiumalatum* (Wall. ex Wight &Arn.) Swingle,(Rutaceae). Journal of Pharmacognosy and Phytochemistry, 4(1), 216-222.

[59] Zhang, H., Deng, L., Yang, M., Min, S., Yang, L., & Zhu, L. (2011). Enhancing Effect of Glycerol on the Tensile Properties of *Bombyx mori* Cocoon Sericin Films Enhancing Effect of Glycerol on the Tensile Properties of *Bombyx mori* Cocoon Sericin Films, (December). https://doi.org/10.3390/ijms12053170

[60] Iwara, I. A., Igile, G. O., Mboso, O. E., Mgbeje, B. I. A., &Ebong, P. E. (2017). Evaluation of phytochemical components from ethyl acetate fraction of *Vernoniacalvoana* using gas chromatography-mass spectrometry analysis and its antioxidants activities. African Journal of Pharmacy and Pharmacology, 11(42), 534-539.

[61] Lee, P. R., Yu, B., Curran, P., & Liu, S. Q. (2010). Kinetics of volatile organic compounds during papaya juice fermentation by three commercial wine yeasts. Nutrition & Food Science.

[62] Meechaona, R., Sengpracha, W., Banditpuritat, J., Kawaree, R., &Phutdhawong, W. (2007). Fatty acid content and antioxidant activity of Thai bananas. Maejo International Journal of science and technology, 1(2), 222-228.

[63] Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., &Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical biology & drug design, 80(3), 434-439.

[64] Sen, T., &Samanta, S. K. (2014). Medicinal plants, human health and biodiversity: a broad review. In Biotechnological applications of biodiversity (pp. 59-110). Springer, Berlin, Heidelberg.

[65] Chong, Y. H., & Ng, T. K. (1991). Effects of palm oil on cardiovascular risk. Med J Malaysia, 46(1), 41-50.

[66] Ruhul, A. M., Kalam, M. A., Masjuki, H. H., Alabdulkarem, A., Atabani, A. E., Fattah, I. R., &Abedin, M. J. (2016). Production, characterization, engine performance and emission characteristics of *Croton megalocarpus* and *Ceibapentandra* complementary blends in a single-cylinder diesel engine. RSC advances, 6(29), 24584-24595.

[67] Selvamangai, G., &Bhaskar, A. (2012). GC–MS analysis of phyto components in the methanolic extract of *Eupatorium triplinerve*. Asian Pacific Journal of Tropical Biomedicine, 2(3), S1329-S1332.

[68] Teres, S., Barceló-Coblijn, G., Benet, M., Alvarez, R., Bressani, R., Halver, J. E., &Escriba, P. V. (2008). Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proceedings of the National Academy of Sciences, 105(37), 13811-13816.

[69] Ismail, G. A., Gheda, S. F., Abo-shady, A. M., & Abdel-karim, O. H. (2020). In vitro potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes. Food Science and Technology, 40(3), 681-691.

[70] Cicero, A. F., &Colletti, A. (2017). Food and plant bioactives for reducing cardiometabolic disease: How does the evidence stack up?. Trends in Food Science & Technology, 69, 192-202.

[71] Turro, N. J., Han, N., Lei, X. G., Fehlner, J. R., & Abrams, L. (1995). Mechanism of dichlorination of n-dodecane and chlorination of 1-chlorododecane adsorbed on ZSM-5 zeolite molecular sieves. A supramolecular structural interpretation. Journal of the American Chemical Society, 117(17), 4881-4893.

[72] Al-Wathnani, H., Ara, I., Tahmaz, R. R., Al-Dayel, T. H., &Bakir, M. A. (2012). Bioactivity of natural compounds isolated from cyanobacteria and green algae against human pathogenic bacteria and yeast. Journal of Medicinal Plants Research, 6(18), 3425-3433.

[73] Singariya, P., Mourya, K. K., & Kumar, P. (2015). Gas Chromatography-Mass Spectrometric Analyses of Acetone extract of Marwar Dhamangrass for bio-active compounds. Plant Archives. 2015a, 15(2), 1065-1074.

[74] Kang, W. Y., Ji, Z. Q., & Wang, J. M. (2009). Composition of the essential oil of *Adiantumflabellulatum*. Chemistry of natural compounds, 45(4), 575.

[75] Cardeal, Z. D. L., Gomes da Silva, M. D. R., & Marriott, P. J. (2006). Comprehensive two-dimensional gas chromatography/mass spectrometric analysis of pepper volatiles. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry, 20(19), 2823-2836.

[76] Kaur, N., Shafiq, N., Negi, H., Pandey, A., Reddy, S., Kaur, H., & Malhotra, S. (2014). *Terminaliaarjuna* in chronic stable angina: Systematic review and meta-analysis. Cardiology research and practice, 2014.

[77] Smritikana Biswas, PranoyMitra. (2017). Comparative studies on the antimicrobial activity of *Terminalia arjuna* and *Aloe vera* against community associated drug resistant *Staphylococcus aureus* in pus from carbuncles of adults. Journal of Innovations in Pharmaceutical and Biological Sciences, 4 (4), 153-157

[78] Desai, D., &Chanda, S. (2014). Pharmacognostic Study and Physicochemical Analysis of Leaves of *Terminalia arjuna*. Pharmacognosy Journal, 6(6), 15-19.

[79] Ahamad, M. S. I., Neetha, K., & Vootla, S. K. (2020). Non-protein Chemical Compounds from Lepidopteran Insect Cocoons. In Natural Materials and Products from Insects: Chemistry and Applications (pp. 137-156). Springer, Cham

[80] Mandal, B. B., Ghosh, B., &Kundu, S. C. (2011). Non-mulberry silk sericin/poly (vinyl alcohol) hydrogel matrices for potential biotechnological applications. International Journal of Biological Macromolecules, 49(2), 125-133. https://doi.org/10.1016/j.ijbiomac.2011.03.015